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From the Department of Microbiology and Immunology,
Veterinary College of Norway, Oslo.

THE OCCURRENCE OF ANTIBODIES AGAINST STAPHYLOCOCCAL DEOXYRIBONUCLEASES IN BLOOD SERA FROM DIFFERENT SPECIES

By
Olav Sandvik

SANDVIK, OLAV: *The occurrence of antibodies against staphylococcal deoxyribonucleases in blood sera from different species.* Acta vet. scand. 1974, *15*, 631—635. — An agar gel method for the detection and separation of antibodies against staphylococcal deoxyribonucleases (DNases) is described. Preliminary tests show that such antibodies occur rather frequently in healthy individuals of various animal species. Two to 3 electrophoretically different fractions of anti-DNases were found in individuals of some species.

deoxyribonucleases; Staphylococcus aureus; antibodies; blood sera.

In 1956 *Cunningham et al.* showed that coagulase-positive, mannitol-hydrolysing strains of Staphylococcus aureus produced a thermostable, calcium-activated deoxyribonuclease (DNase). This enzyme has become an interesting factor in the complex of extracellular biologically active compounds produced by pathogenic strains of staphylococci. Properties of the enzyme have been reviewed by *Abramson* (1972). Due to the correlation of the enzyme production with other pathogenic criteria, primarily coagulase production, testing for DNase has been proposed as a convenient aid in the identification of pathogenic strains of S. aureus. Improved methods for the demonstration of the enzyme (*Lachica et al.* 1971, *Barry et al.* 1973) have been used routinely in many diagnostic laboratories.

Scharmann & Blobel (1970) demonstrated different antigenic properties of DNases from different strains of staphylococci by agar gel precipitation.

The attention paid to the staphylococcal DNases as a diagnostic and pathogenic criterion emphasizes the importance of gaining more knowledge with regard to the influence of these

enzymes on the infected host organism. The present paper deals with the demonstration of DNase antibodies by means of enzyme neutralization based on a special agar-gel procedure.

MATERIALS AND METHODS

Strains. Eight strains of S. aureus were used. Two strains (NVH* 219 and NVH 588) were isolated from human abscesses, 1 (NVH 513) from sheep mastitis, 1 (NVH 19) from bovine mastitis, 1 (NVH 729) from pig abscess, 1 (NVH 16) from horse abscess, 1 (NVH 97/74) from dog abscess, and 1 (NVH 937) from a cockerel liver. All strains were distinctly different with regard to phage types.

Sera. Altogether 35 sera obtained from assumed healthy individuals of goat (5 sera), cattle (9 sera), horse (11 sera), dog (5 sera) and pig (5 sera) were examined. The samples were picked randomly, but records were made as to whether the animals were adults or younger individuals.

Enzyme-containing material. The production of DNases for serological examinations was made by growing the organisms in 5 ml amounts of nutrient broth (Difco) for 24 hrs. at 37°C.

Agar medium for the detection of DNases. Toluidine blue deoxyribonucleic acid agar (TDA) (*Lachica et al.* 1971) was used. The medium, which alone does not support growth of staphylococci, was poured into glass trays to a depth of 2 mm and allowed to solidify.

Determination of DNase inhibitors. Filter paper strips moistened with blood serum were placed on the surface of the TDA agar. After 19 hrs., these strips were removed, and strips moistened with staphylococcal broth culture were placed at right angles to the serum. Inhibition of enzyme activity occurred as interruptions of the otherwise pink zones along the DNase containing strips, or by a greater or lesser narrowing of these zones (Fig. 1 a). The test was made semiquantitative by using serial 2 fold dilutions of the serum samples, and determining the highest dilution exhibiting visual inhibition of the enzyme reaction.

By a so-called electrophoretic DNase inhibition test the sera were subjected to paper electrophoresis before being brought into contact with the enzyme-containing broth. A type 3276 BN (LKB Stockholm) apparatus was used with Schleicher and Schüll No. 2043 bmgl. paper, and 0.05 M phosphate buffer, pH 6.5. The sera were applied in 8—10 µl amounts and run at 120 v for 16 to 18 hrs. The wet paper strips were then transferred immediately to the surface of TDA-medium. After incubation at 37°C for 2—3 hrs., the strips were removed and replaced by narrow (0.5 to 0.8 cm) strips of filter paper that had been immersed in the enzyme-containing culture to be tested. Two strips were placed parellel within the 4 cm broad band of the electrophoresis paper for 2 to 18 hrs. at 37°C depending on the amount of

* NVH: The culture collection at the Department of Microbiology and Immunology, Veterinary College of Norway.

development desired. The typical pink zones that occurred along the enzyme-containing strips were interrupted or narrowed at certain places corresponding to the location of the inhibiting compounds (Fig. 1 b). For comparison replicate electrophoresis strips were stained with amido black (*Science Tools* 1958).

Precipitation of immunoglobulins. Immunoglobulins were precipitated from 1 serum of each species by the method of *Stelos* (1967).

RESULTS

The effect of staphylococcal DNases in the TDA medium occurred distinctly as bright pink haloes in contrast to the otherwise blue medium. The inhibitory compounds in serum were easy to detect (Fig. 1), and the degree of visual inhibition correlated with the amounts of inhibitors in the samples. The number of sera exhibiting inhibitory effects on the staphylococcal DNases was very high. Only 1 serum each from goat, cattle, horse, and pig, which all originated from individuals less than

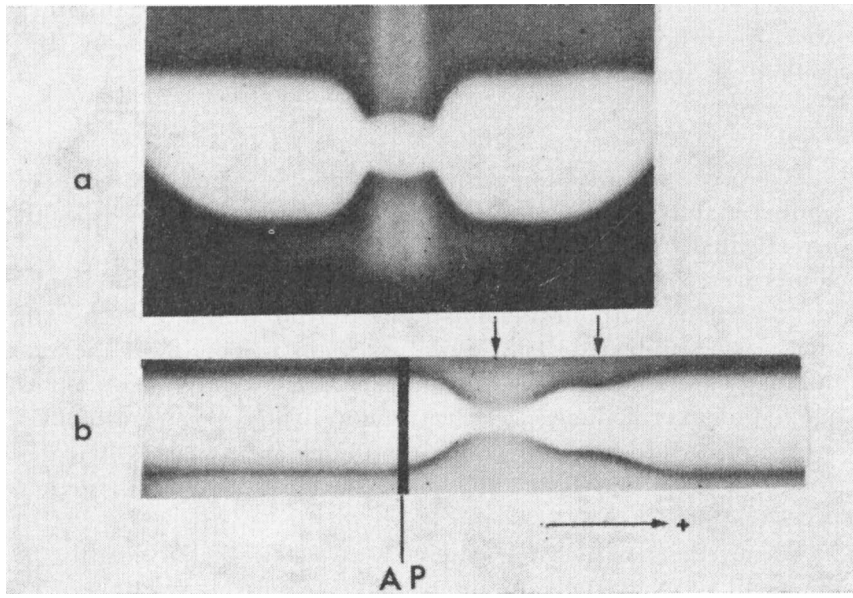

Figure 1 a. Inhibition of staphylococcal DNase activity in TDA-agar caused by serum from horse (applied vertically).

b. Effect of the same horse serum in the electrophoretical inhibition test showing 2 inhibitory factors (arrows) in the immunoglobulin area. The electrophoresis was carried out in 0.05 M phosphate buffer at pH 6.5 for 18 hrs. at 120 v. AP: Line of application.

3 months of age, and 4 of 5 sera from dog were completely negative with respect to inhibitory compounds. By the semiquantitative test the titres of the different sera varied between 1:8 and 1:256.

Generally, the sera showing inhibitory effects equally inhibited the DNases produced by most of the staphylococcal strains tested. Exceptions were the dog strain that was only affected by one single dog serum, and the cockerel strain that was not inhibited by any serum tested. The electrophoretic inhibition test demonstrated up to 3 different inhibitory factors in goat, cattle, and horse and up to 2 in pig and dog all with distinctly different electrophoretic migratory properties (Fig. 1 b). All the strains, except those originating from dog and cockerel, produced DNases that exhibited apparently identical enzymoserological patterns towards the sera used. All the inhibitors were located within the area of the immunoglobulins as judged from the electrophoretic migration rate compared with replicate stained electrophoregrams, and from the fact that the inhibitors were quantitatively precipitated as immunoglobulins by the method of *Stelos* (1967).

DISCUSSION

The experiments indicate that inhibitory serum components against staphylococcal DNases are very common in most species investigated. Thus, most samples from the randomly picked adults were positive. On the other hand, individuals, young ones in particular, without demonstrable serum inhibitors exist in all the species tested. The inhibitors seem to belong to the immunoglobulins and should be considered as acquired antibodies against deoxyribonuclease. Electrophoretically these antibodies seem to fall into 2—3 different immunoglobulin classes or categories in some species. This question, however, needs further investigation.

As all the positive individuals were classified as healthy, it seems likely that the immunological mechanism is rather sensitive to staphylococcal DNases. Thus, the comparatively frequent superficial confrontation with more or less ubiquitous strains of S. aureus in some individuals may be sufficient to induce a significant immune response against DNases.

The examinations indicate that strains isolated from various animal species, and belonging to different phage types, may be-

have very similarly enzymo-serologically, while others (for instance the strain from dog and cockerel) may be completely different.

The enzymo-serological procedure described seems to be suited for the study of the staphylococcal influence on the host and for the comparison of different categories of staphylococci. The correlation of the occurrence of thermostable DNases with the pathogenic properties of these microorganisms indicates that the DNases may be important factors in a possible model for the study of staphylococcal infections.

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SAMMENDRAG

Forekomst av antistoffer mot stafylokokk-deoxyribonukleaser i blodsera fra forskjellige arter.

En har beskrevet en spesiell agargel-metode for påvisning og separasjon av antistoffer mot stafylokokk-deoxyribonuklease (DNase). Preliminære undersøkelser viser at slike antistoffer forekommer forholdsvis hyppig hos friske individer av forskjellige dyrearter. To til tre elektroforetisk forskjellige fraksjoner av anti-DNaser ble påvist hos individer fra enkelte arter.

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Reprints may be requested from: Olav Sandvik, Department of Microbiology and Immunology, Veterinary College of Norway, PO Box 8146, Oslo Dep., Oslo 1, Norway.